

Yarrowia lipolytica as a Phosphate-Solubilizing Yeast for Plant Growth Promotion

Gilma Alves da Silva

`gilma.silva@ufv.br`

Universidade Federal de Viçosa

Paula Meire Soares Gomes

Universidade Federal de Viçosa

Henrique Pereira Guimarães

Universidade Federal de Viçosa

Felipe Campos Silva

Universidade Federal de Viçosa

Amanda de Jesus Barbosa

Universidade Federal de Viçosa

Juscimar da Silva

Brazilian Agricultural Research Corporation

Mauricio Dutra Costa

Universidade Federal de Viçosa

Nairam Félix de Barros

Universidade Federal de Viçosa

Rafael da Silva Teixeira

Universidade Federal de Viçosa

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Abstract

Background

Phosphorus (P) availability is a major constraint to crop production in highly weathered tropical soils, highlighting the need for sustainable alternatives to improve P use efficiency. This study evaluated the plant growth-promoting potential of *Yarrowia lipolytica* through *in vitro* characterization and greenhouse experiments.

Results

The yeast exhibited high calcium phosphate solubilization (71.2%), lower solubilization of iron and aluminum phosphates, and the ability to mineralize phytate (6.75 mg L^{-1}). Acidification of the culture medium, together with high acid and alkaline phosphatase activities, indicated that organic acid production and phosphatase secretion were the main mechanisms involved in P mobilization. The yeast also produced the phytohormones indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA), supporting an additional mechanism for plant growth promotion. Under greenhouse conditions, inoculation significantly increased plant height, stem diameter, fruit number, and fruit yield of Micro-Tom tomato, with the greatest responses observed at the highest inoculation dose. In contrast, no significant effects were detected in rice, bean, or maize grown in a highly weathered, nutrient-poor soil, although inoculated plants consistently showed a positive growth trend.

Conclusions

Overall, *Y. lipolytica* exhibits multiple plant growth-promoting traits, particularly those related to phosphorus mobilization and phytohormone production, demonstrating its potential as an agricultural bioinput. However, its effectiveness in promoting plant growth depends on substrate fertility, the availability of compatible phosphorus sources, and inoculation dose.

1 Introduction

Sustainable agriculture has become a pressing necessity in the face of increasing environmental challenges and the growing global demand for food. It involves practices that ensure long-term productivity while preserving soil health and integrity, minimizing environmental impacts, and guaranteeing food security. In this context, reducing the use of chemical inputs, especially synthetic fertilizers, stands out as a central aspect of sustainability, as these products can lead to soil degradation, water pollution through eutrophication, and biodiversity loss [1]. At the same time, the use of biological inputs, such as plant growth-promoting microorganisms and biofertilizers, has emerged as a promising alternative, offering both environmental and economic benefits while supporting healthy plant development and improving nutrient use efficiency in the soil [2].

Phosphorus (P) availability in soil results from a complex interplay of physical, chemical, and biological processes, influenced by the forms of P present and environmental conditions. In soil, P can occur in inorganic forms, mainly as calcium, iron, and aluminum phosphates, or in organic forms associated with plant and microbial residues [3, 4]. The inorganic fraction typically represents 35–70% of total P, whereas the organic fraction ranges from 30–65% [5]. However, only a small portion of total P is readily available to plants, often less than 0.1% [6]. In highly weathered tropical soils, much of the total P occurs in poorly available forms, strongly adsorbed to iron and aluminum oxides or precipitated with Fe^{3+} , Al^{3+} , and Ca^{2+} . Under these conditions, the labile P fraction limits the agronomic efficiency of phosphate fertilizers, effectively turning the soil into a P sink [7, 8].

Phosphorus-solubilizing microorganisms (PSMs) play a crucial role in converting poorly available P into forms assimilable by plants [3, 4]. The solubilization of inorganic phosphate primarily occurs through the production of low-molecular-weight organic acids, such as citric, gluconic, oxalic, malic, and 2-ketogluconic acids, which promote local acidification, displacement of adsorbed phosphates, and chelation of metal cations (Ca^{2+} , Fe^{3+} , and Al^{3+}) [3, 4]. In addition, many PSMs are capable of mineralizing organic P through the secretion of extracellular phosphatases and phytases, which hydrolyze compounds such as phytates, phosphoesters, and phospholipids, releasing plant-assimilable phosphorus [9–11]. Thus, PSMs contribute both to the mobilization of inorganic P and the mineralization of organic P, enhancing nutrient availability in the soil.

In turn, the yeast *Yarrowia lipolytica* is a dimorphic, heterothallic, non-pathogenic ascomycete notable for its lipolytic and proteolytic capabilities [12]. In terms of key characteristics, *Y. lipolytica* is an obligate aerobe with broad tolerance to physicochemical conditions, capable of growing on a variety of substrates, including hydrophobic and hydrophilic carbon sources. Despite being commercially exploited for over 70 years in citric acid production [1], the use of *Y. lipolytica* as an agricultural bioinput remains largely underexplored. Although this yeast has been extensively investigated for industrial biotechnology applications, only a limited number of studies have evaluated its potential as a plant growth-promoting microorganism. Consequently, important aspects of its mechanisms of action, including phosphorus solubilization, phosphorus mineralization, and its effects on plant development, remain poorly understood, highlighting a significant knowledge gap in agricultural research.

It was hypothesized that *Yarrowia lipolytica* is capable of mobilizing poorly available phosphorus through mechanisms of inorganic P solubilization and organic P mineralization, thereby increasing P availability to plants. Additionally, the plant growth-promoting traits of this yeast are expected to enhance plant development, with the magnitude of these effects varying according to the fertility conditions of the growth substrate.

Therefore, considering the challenges of P availability in highly weathered tropical soils and the biochemical potential of *Y. lipolytica*, the development of biofertilizers based on this yeast offers a promising strategy to increase phosphorus use efficiency, reduce reliance on mineral fertilizers, and promote sustainable agricultural production. Thus, the objective of this study was to characterize the

potential of *Yarrowia lipolytica* as a plant growth promoter, integrating *in vitro* assays of mechanisms associated with growth promotion and *in vivo* assays with plants grown in substrates under varying fertility conditions.

2 Material and Methods

2.1 Evaluation of *in vitro* plant growth-promoting (PGP) traits

2.1.1 Qualitative and quantitative estimation of inorganic phosphate (P) solubilization and organic P mineralization

The qualitative capacity of yeast for phosphate solubilization and phytate mineralization was evaluated by halo formation on solid medium. For phosphate solubilization, modified National Botanical Research Institute's phosphate growth medium (NBRIP) [14] was used, containing: 10 g glucose; 0.1 g $(\text{NH}_4)_2\text{SO}_4$; 0.2 g KCl; 5 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$; 0.25 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 5 g $\text{Ca}_3(\text{PO}_4)_2$; and 15 g agar, with the pH adjusted to 7 per liter. For phytate mineralization, modified NBRIP medium [14] contained: 10 g glucose; 0.5 g $(\text{NH}_4)_2\text{SO}_4$; 0.2 g NaCl; 0.2 g KCl; 0.1 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 0.001 g MnSO_4 ; 0.2 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$; 1.9 g MgCl_2 ; 5 g Sodium Phytate; and 18 g agar, with the pH adjusted to 7.0. Tests were conducted using two distinct inoculation approaches: i) either by depositing a 10 μL drop of the yeast suspension directly onto the medium or ii) by incorporating sterilized *Glycine max* cv. MiniMax seeds, previously inoculated by immersion, to verify the interaction. The plates were incubated in a BOD incubator at 28°C for 12 days in half-sheets or full sheets.

Quantification of yeast phosphorus solubilization capacity was performed in 50 mL of modified liquid NBRIP medium [14] (as described in the previous section, without agar and varying the P source). The yeast was tested for its ability to solubilize inorganic calcium [$\text{Ca}_3(\text{PO}_4)_2$], iron (FePO_4), and aluminum (AlPO_4) phosphates, as well Araxá (13.9% P), Bayóvar (11.8% P), Catalão (16.2% P), and Patos (14.4% P) rock phosphates. The medium was inoculated with 5 mL of microbial suspension and continuously agitated at 150 rpm for 7 days at 28°C. After the seventh day, the material was filtered and evaluated using the colorimetric reaction [15] at 725 nm, by subtracting the soluble phosphorus in the treatments from that contained in the control sample. The pH and titratable acidity values were also measured.

To quantify phytate mineralization, yeast was grown in YPD medium for 24 h at 150 rpm and 28°C. Aliquots of 1000 μL were transferred to a conical tube containing 20 mL of modified NBRIP supplemented with 40 g L^{-1} of glucose and 1 g L^{-1} of sodium phytate as a source of phosphorus. After incubating for nine days at 120 rpm and 28°C, the material was filtered and evaluated using the colorimetric reaction [15] at 725 nm, by subtracting the soluble phosphorus in the treatments from that contained in the control sample. The pH and titratable acidity values were also measured.

2.1.2 Acid and alkaline phosphatase productions

The yeast was inoculated in triplicate into NBRIP medium [14] and incubated at 30°C and 150 rpm. After 24 h of incubation, the samples were centrifuged at 3,350×g for 15 min. Subsequently, 150 µL of the supernatant were processed according to Nahas [16] and measured at 400 nm. A standard curve was prepared using a p-nitrophenol (pNP) solution with concentrations ranging from 0 to 25 µg mL⁻¹.

2.1.3 Indole-3-acetic acid (IAA) and Indole-3-butyric acid (IBA) production for auxin biosynthesis

Indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) production was determined by a colorimetric method based on Salkowski's reagent [17, 18]. The yeast was cultured in YPD medium supplemented with 5 mM L-tryptophan for 96 hours at 150 rpm. After this period, triplicates were analyzed by centrifugation at 10,000 rpm for 5 minutes. Salkowski solution (1:1) was added to the supernatant until color development. Absorbance was measured at 530 nm for IAA and 480 nm for IBA.

2.1.4 Siderophores production assay

Siderophore production by the yeast isolate was evaluated using two approaches. For catechol- and hydroxamate-type siderophores, three 5 µL drops of culture supernatant obtained from TSB medium (incubated at 28°C for 24 h) were placed equidistantly on nutrient agar plates (20 mL). The plates were incubated at 28°C for 16 h, followed by the addition of a CAS agar overlay and further incubation for 4 days at 28°C. The formation of pink, orange, or light-yellow halos indicated the production of catechol, hydroxamate, or carboxylate siderophores, respectively [19]. For pyoverdine-type siderophores, the same procedure with 5 µL drops of culture supernatant was performed on King B agar medium (20 g L⁻¹ proteose peptone, 1.5 g L⁻¹ K₂HPO₄, 1.5 g L⁻¹ MgSO₄·7H₂O, 10 mL L⁻¹ glycerol, and 15 g L⁻¹ bacteriological agar). The plates were incubated in a BOD chamber for 5 days at 28°C, and fluorescence under UV light (366 nm) to confirm pyoverdine production [20].

2.1.5 Exopolysaccharide (EPS) production assay

The assessment of EPS production followed the protocol described by Paulo *et al.* [21]. Yeast suspensions (5 µL) were inoculated onto sterile filter paper discs (5 mm diameter, Whatman 42) placed on solid medium (pH 7.5) containing sucrose (50 g L⁻¹), yeast extract (20 g L⁻¹), MgSO₄ (20 g L⁻¹), NaCl (0.01 g L⁻¹), FeSO₄ (0.01 g L⁻¹), MnSO₄ (0.01 g L⁻¹), CaCl₂ (0.02 g L⁻¹), K₂HPO₄ (20 g L⁻¹), and agar (18 g L⁻¹). Plates were incubated at 30°C for 24 h. EPS production was evaluated by the formation of mucoid colonies around the discs, with confirmation obtained through the alcohol precipitation method: a portion of the mucoid substance was mixed with 2 mL of absolute ethanol, where the appearance of a precipitate indicated a positive result, while turbidity indicated a negative result.

2.1.6 Ammonium (NH₄⁺) production assay

The yeast was inoculated at an optical density of 0.1 into tubes containing 10 mL of a solution with 1% peptone and 0.5% NaCl, and incubated at 28°C with constant shaking at 150 rpm for 48 hours. Then, 200 µL of the supernatant were mixed with 1 mL of Nessler's reagent and distilled water to a final volume of 8.5 mL. Absorbance was measured at 450 nm [22].

2.1.7 Hydrogen cyanide (HCN) production assay

HCN production by the yeast isolate was assessed on King B agar plates (20 mL). A quantitative filter paper strip (JP42 Quanty®, 1 × 3 cm, 8 µm porosity), previously sterilized at 180°C for 2 h, was impregnated with a 0.5% picric acid solution in 2% sodium carbonate (Na₂CO₃) and fixed to the inner surface of the Petri dish lid. The inoculated plates were sealed with PVC film and incubated in a BOD chamber at 28°C for 5 days. A color change of the filter paper from yellow to orange, brown, or reddish-brown indicated HCN production [23].

2.1.8 Cellulase production assay

Cellulase production was verified by inoculating the yeast into CMC agar medium (2.0 g L⁻¹ carboxymethylcellulose, 2.0 g L⁻¹ NaNO₃, 1.0 g L⁻¹ K₂HPO₄, 0.5 g L⁻¹ KCl, 0.5 g L⁻¹ MgSO₄, 0.2 g L⁻¹ peptone, and 17.0 g L⁻¹ agar), followed by incubation in a B.O.D. chamber at 28°C for 48 hours. After growth, an iodine solution (2 g KI and 1 g iodine dissolved in 300 mL of distilled water) was added to the colonies and allowed to react for 3 to 5 minutes. The formation of halos around the colonies indicated CMC degradation and, consequently, cellulase production by the isolates [24].

2.1.9 Chitinase production assay

Chitinase production was verified in Petri dishes containing 20 mL of culture medium for chitinolytics (1.0 g L⁻¹ (NH₄)₂SO₄, 0.2 g L⁻¹ KH₂PO₄, 1.6 g L⁻¹ K₂HPO₄, 0.2 g L⁻¹ MgSO₄·7H₂O, 0.1 g L⁻¹ NaCl, 0.01 g L⁻¹ FeSO₄·7H₂O, 0.02 g L⁻¹ CaCl₂·2H₂O and 15 g L⁻¹ agar). The medium was supplemented with 1% (w v⁻¹) colloidal chitin prepared according to Skujins *et al.* [25]. Three 5 µL microdroplets of the supernatant from a culture grown in TSB medium at 28°C for 24 h were placed equidistantly on the surface of the medium in the Petri dish. The plates were incubated in B.O.D. for 96 h at 28°C. The formation of a translucent halo around the colonies would indicate that they were chitinase producers.

2.2 Evaluation of *in vivo* plant growth-promoting (PGP)

2.2.1 Yeast inoculation in maize, rice, and beans grown in a nutrient-poor soil

To evaluate the growth-promoting effect of the yeast on plants in a nutrient-poor soil, an experiment in a greenhouse was performed following a 2 (without and with yeast inoculation) × 3 [maize (*Zea mays* L., cv. UFVM100), rice (*Oryza sativa* L.), and common bean (*Phaseolus vulgaris* L., cv. Marte)] factorial, using a randomized complete block design with four replicates. Seeds of maize, rice, and common bean were obtained from seed stocks maintained at the Department of Agronomy, Universidade Federal de Viçosa

(UFV), Viçosa, Minas Gerais, Brazil. Cultivar information was not available for rice. Pots (5-L) were filled with clay soil classified as Typic Hapludox [26] (pH: 5.08; P: 0.3 mg dm⁻³; K⁺: 17.0 mg dm⁻³; Ca²⁺: 0.3 cmol_c dm⁻³; Mg²⁺: 0.17 cmol_c dm⁻³; Al³⁺: 0.97 cmol_c dm⁻³; H + Al: 5.0 cmol_c dm⁻³; SOM: 2.37 dag kg⁻¹; P-rem: 13.1 mg L⁻¹). No fertilization was performed on the soil, so that the growth-promoting effect of the yeast could be evaluated under nutrient-limiting conditions, where microbial activity would play a key role in nutrient mobilization and availability to plants.

Yeast growth was carried out in YPD medium under constant agitation (150 rpm) at 28°C for 24 hours. Seeds of different plants were surface-sterilized and inoculated by immersion in the yeast suspension with 10⁸ CFU mL⁻¹. The inoculated seeds were sown in the soil, and the plants were maintained under greenhouse conditions. Fifteen days after sowing, the plants received a second inoculation with 4 mL of the corresponding suspension (10⁸ CFU mL⁻¹). After 30 days, plant height (cm) and stem diameter (mm), and after 60 days the shoots and roots dry matter mass (g pot⁻¹) were measured.

2.2.2 Yeast inoculation at different doses in Micro-Tom tomato grown in a nutrient-rich substrate

To evaluate the role of the yeast in promoting the growth of plants in a nutrient-rich substrate, an experiment in a greenhouse was conducted using *Micro-Tom* tomato (*Lycopersicon esculentum* cv. *Micro-Tom*) in which the seedlings were subjected to five volumes (0, 2, 4, 8, and 16 mL) of yeast suspension (10⁸ CFU mL⁻¹). Seeds were obtained from a Micro-Tom seed stock maintained by the research group at the Department of Soil and Plant Nutrition, Universidade Federal de Viçosa (UFV), Viçosa, Minas Gerais, Brazil. Micro-Tom was originally developed at the University of Florida, USA. The experiment was conducted using a randomized complete block design with four replicates. The yeast suspension was applied at transplanting and again 15 days later.

Yeast cultures were grown in YPD medium under constant agitation (150 rpm) at 28°C for 24 hours. The inoculum was prepared based on optical density readings at 600 nm, adjusted to an absorbance of 0.600 (~ 10⁸ CFU mL⁻¹). Seedlings were transplanted into 3-L pots filled with commercial substrate (Tropstrato HT Hortaliças – pH: 5.04; P: 231.9 mg dm⁻³; K⁺: 1007 mg dm⁻³; Ca²⁺: 9.92 cmol_c dm⁻³; Mg²⁺: 9.35 cmol_c dm⁻³; Al³⁺: 0.06 cmol_c dm⁻³; H + Al: 12.2 cmol_c dm⁻³; SOM: 7.22 dag kg⁻¹; P-rem: 39.9 mg L⁻¹), and inoculated with the corresponding yeast suspension volumes. The use of a substrate with high nutrient availability aimed to verify whether the yeast could still exert growth-promoting effects even under non-limiting fertility conditions.

Plant height (cm) was measured with a ruler, and stem diameter (mm) was determined using a caliper at 15, 22, and 28 days after transplanting. The experiment was conducted until 35 days, at which shoots and roots dry matter mass (g pot⁻¹), number of fruits, and total fruit weight (g) were measured.

2.3 Statistical analysis

The experiments on phosphate solubilization quantification, phytate mineralization, and growth promotion in maize, rice, common bean, and MicroTom tomato were subjected to tests of homogeneity and normality. Once these assumptions were met, the data were analyzed using ANOVA at a 5% probability level. When significant effects were observed, means were compared using Tukey's test at a 5% probability level. The data relating to the analysis of the set of corn, beans and rice without and with inoculation were compared using a contrast test at 10% significance. Additionally, a principal component analysis (PCA) was performed for the *in vivo* experiment with MicroTom tomato to evaluate the joint variation among the measured traits.

3 Results

3.1 Evaluation of *in vitro* plant growth-promoting (PGP) traits

The qualitative test for phosphate solubilization (calcium phosphate) showed a very subtle formation of halos (Fig. 1a), but they were not visible in the soybean MiniMax inoculation. However, both cases showed positive results in the phytate mineralization assay (Fig. 1b-f). Soybeans inoculated with yeast began germinating on the second day, while those not inoculated germinated from the seventh day onwards.

In the quantitative test for inorganic phosphate solubilization, after seven days, the yeast solubilized 71.2% of calcium phosphate (P-Ca), 14.0% of Bayóvar RP, 7.2% of Araxá RP, 5.3% of Patos RP, 5.2% of Catalão RP, 3.6% of aluminum phosphate (P-Al), and 3.5% of iron phosphate (P-Fe) (Fig. 2a). The final pH of the media containing the different phosphates ranged from 3.17 to 3.87. Higher values were observed in Bayóvar (3.87), P-Ca (3.66), Araxá (3.53), and Catalão (3.52) RPs compared to the uninoculated control, while P-Al (3.26) and P-Fe (3.17) showed lower values (Fig. 2b). Despite the close pH values, titratable acidity was statistically higher only in P-Ca (53.31 g L^{-1}), while the others showed an average of 13.31 g L^{-1} (Fig. 2c).

The yeast mineralized approximately 6.75 mg L^{-1} of phytate after nine days, with the culture medium showing a pH of around 3.23 compared to 4.51 in the control, and a titratable acidity of $15.44 \text{ mmol L}^{-1}$, compared to 0.97 in the control. The activities of phosphatase enzymes were $24.39 \text{ } \mu\text{g pNP mL}^{-1} \text{ h}^{-1}$ (acidic) and $21.70 \text{ } \mu\text{g pNP mL}^{-1} \text{ h}^{-1}$ (alkaline). In the auxin production assay, the yeast produced on average $27.28 \text{ } \mu\text{g mL}^{-1}$ of IAA and $34.95 \text{ } \mu\text{g mL}^{-1}$ of IBA, after four days. In contrast, no positive results were obtained for siderophore, EPS, NH_4^+ , HCN, cellulase, or chitinase production (Table 1).

Table 1
In vitro plant growth-promoting characteristics of *Y. lipolytica*.

Characteristics	Results
Phytate mineralization (mg L ⁻¹)	6.75
Acid phosphatase (µg pNP mL ⁻¹ h ⁻¹)	24.39
Alkaline phosphatase (µg pNP mL ⁻¹ h ⁻¹)	21.70
Indole acetic acid (IAA) (µg mL ⁻¹)	27.28
Indole butyric acid (IBA) (µg mL ⁻¹)	34.95
Siderophores	-
Exopolysaccharide (EPS)	-
Ammonium (NH ₄ ⁺)	-
Hydrogen cyanide (HCN)	-
Cellulase	-
Chitinase	-

The negative (-) signs means negative results for each PGP characteristic.

3.2 Evaluation of *in vivo* plant growth-promoting (PGP)

At 30 days after sowing, rice, bean, and maize plants showed no significant differences in response to yeast inoculation when evaluated individually or jointly (Fig. 3). At 60 days, dry matter measurements for shoot, root, total, and relation between root and shoot did not differ significantly between inoculated and non-inoculated plants (Fig. 4). Although these differences were not statistically significant, inoculated plants tended to exhibit slightly higher dry matter values than non-inoculated plants (Fig. 5).

In the MicroTom tomato experiment, for plant height, at 28 days, a significant difference ($p = 0,0245$) was detected between the non-inoculated treatment (11.44 cm) and inoculation with 16 mL (19.99 cm), corresponding to an increase of 6.46 cm between 15 and 22 days and 5.25 cm between 22 and 30 days, and in stem diameter increment between 21 and 28 days ($p = 0.0135$), with 16 mL inoculation resulting in an increase of 0.66 mm compared to only 0.18 mm in the non-inoculated treatment (Fig. 6). Significant effects were also detected for fruit number ($p = 0.0125$; 16 mL = 10.75 vs. 0 mL = 4.38) and total fruit weight ($p = 0.0282$; 16 mL = 48.6 g vs. 0 mL = 20.24 g), as shown in Figs. 7e and 7f. No significant differences were observed for dry matter mass results. (Figs. 7a-7d). Representative visual differences between inoculated and uninoculated plants during the flowering and fruiting stages are shown in Fig. 8.

Principal component analysis (PCA) revealed that the first two components jointly explained 75.83% of the total variance, with PC1 accounting for 52.78% and PC2 for 23.06% (Fig. 9). PC1 was positively correlated with growth and total biomass variables, including TDMM (0.318), RDMM (0.317), SDMM (0.317), TFW (0.283), and AFW (0.283), indicating that this axis primarily reflects the overall vegetative development of the plants. Individuals corresponding to higher inoculation volumes (8 and 16 mL) exhibited positive PC1 scores (2.21 and 2.56, respectively), demonstrating greater biomass accumulation, whereas the non-inoculated treatment (0 mL) showed negative values (-5.11), indicating reduced growth.

PC2 explained 23.06% of the variance and was mainly influenced by initial morphometric variables and biomass allocation ratios, such as H1 (-0.395), D1 (-0.447), H2 (0.326), HI2 (0.327), and RSR (-0.235), suggesting that this component captures differences related to initial plant morphology and biomass partitioning between root and shoot. Intermediate inoculation treatments (2 and 4 mL) had negative PC2 scores (-1.83 and -1.73, respectively), indicating reduced initial height development and a lower root-to-shoot ratio, whereas the 16 mL treatment showed positive values (2.94), reflecting superior morphometric characteristics.

4. Discussion

Plant growth promotion by microorganisms can occur through different mechanisms, including nutrient mobilization, phytohormone production, and the induction of physiological responses that enhance plant tolerance to biotic and abiotic stresses [27, 28]. In this context, the present results demonstrate that *Yarrowia lipolytica* has the potential to act through multiple pathways simultaneously by combining phosphorus mobilization with the synthesis of bioactive compounds capable of stimulating plant development. In addition, previous reviews have highlighted the remarkable metabolic versatility of *Y. lipolytica*, its tolerance to adverse environmental conditions, and its potential use as both a biofertilizer and a biocontrol agent [28–30].

The *in vitro* characterization revealed a high capacity for calcium phosphate solubilization, whereas iron and aluminum phosphates were solubilized to a much lesser extent. This difference suggests a strong influence of phosphate source chemistry on solubilization efficiency, since microbial phosphate solubilization depends on mineral reactivity, medium composition, and the metabolites produced by the microorganism [31]. The marked decrease in medium pH, together with the increase in titratable acidity, indicates that organic acid production was the main mechanism involved in phosphate solubilization. This process is commonly associated with medium acidification and the subsequent dissolution of poorly soluble phosphate compounds. Acidification particularly favors the dissolution of calcium-bound phosphates, increasing phosphorus availability for plant uptake. These findings agree with previous soil–plant studies in which *Y. lipolytica* improved phosphorus utilization when combined with a compatible phosphate source and the arbuscular mycorrhizal fungus *Glomus deserticola* [30].

In addition to mobilizing inorganic phosphorus, the yeast was able to mineralize organic phosphorus from phytate. This result is directly associated with the production of acid and alkaline phosphatases detected during the biochemical characterization, indicating the ability to access less readily available organic phosphorus pools. The capacity to utilize organic phosphorus enhances the agronomic potential of the microorganism, since a substantial proportion of soil phosphorus is associated with organic compounds and depends on microbial activity to become available for plant uptake. The metabolic versatility of *Y. lipolytica* regarding phosphorus metabolism has also been reported in physiological studies demonstrating efficient phosphorus uptake systems over a wide pH range, although the final agronomic response depends on the predominant phosphorus form in the soil [32].

Another important mechanism identified was the production of the phytohormones indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA). These auxins play a fundamental role in cell division, tissue elongation, and root development [33]. Auxin production by plant growth-promoting yeasts has been recognized as one of the main mechanisms responsible for stimulating plant growth, even under conditions where nutrient limitation is not the predominant factor [27, 28]. Therefore, the ability of *Y. lipolytica* to synthesize growth regulators suggests that its benefits extend beyond mineral nutrition, involving physiological changes that directly promote plant growth and development.

This hypothesis is supported by the results obtained with Micro-Tom tomato plants. Although the experiment was conducted in a nutrient-rich substrate, where nutritional limitation was minimal, positive responses to inoculation were observed, particularly at the highest inoculation doses. Recent studies have shown that plant growth-promoting microorganisms can modify plant physiological parameters even under fertile conditions through phytohormone production, metabolic modulation, and improved physiological efficiency [34]. Specifically, inoculation with *Y. lipolytica* increased fresh biomass by 11.5% and dry biomass by 7.1% in *Salvinia auriculata*, in addition to increasing photosynthetic pigment content under certain experimental conditions [35]. In another study with the same plant species, inoculation with *Y. lipolytica* increased fresh biomass by 47% and dry biomass by 27% compared with non-inoculated plants [36]. In the present study, increasing the inoculant dose significantly enhanced plant height, stem diameter, and fruit production. Principal component analysis confirmed this trend by associating the highest inoculation doses with variables related to vegetative growth, biomass production, and yield.

Considering the high nutrient availability of the substrate, these responses are unlikely to be explained solely by increased phosphorus availability. Instead, they are more likely associated with the production of plant growth-promoting metabolites, particularly phytohormones [27, 28, 34]. The *in vitro* production of IAA and IBA supports this interpretation. However, the increase in shoot dry matter suggests that additional regulatory compounds may also be involved. Although cytokinin production was not evaluated in the present study, the possibility that *Y. lipolytica* produces cytokinins or other bioactive metabolites cannot be excluded and deserves further investigation.

In contrast to the results obtained with tomato, rice, bean, and maize did not show statistically significant differences among treatments, although inoculated plants consistently exhibited a tendency

toward greater growth and biomass production. These experiments were conducted in a highly weathered soil without fertilization or previous agricultural cultivation, conditions that severely limited nutrient availability and may also have restricted the activity of *Y. lipolytica*. Because this yeast preferentially solubilizes calcium phosphates, the low availability of this phosphorus form, together with the predominance of iron and aluminum oxides in the soil, may have limited the expression of this mechanism, explaining the reduced response observed in these crops.

Another important aspect is the possible influence of inoculation dose on plant response. While rice, bean, and maize received only 4 mL of inoculant, the Micro-Tom experiment demonstrated that the best responses occurred at the highest inoculation doses. The progressive improvement observed with increasing inoculation volume suggests that higher microbial populations may be necessary to ensure effective colonization and consistent expression of plant growth-promoting mechanisms. Therefore, higher inoculation doses than those used in the grain experiments might produce more pronounced responses, particularly under severe nutrient-limited conditions.

5. Conclusion

Overall, the results demonstrate the potential of *Yarrowia lipolytica* as an agricultural bioinput based on multiple *in vitro* plant growth-promoting traits. The yeast solubilized different inorganic P sources, with efficiencies of 71.2% for Ca-P, 14.0% for Bayóvar RP, 7.2% for Araxá RP, 5.3% for Patos RP, 5.2% for Catalão RP, 3.6% for Al-P, and 3.5% for Fe-P. *Y. lipolytica* also exhibited acid and alkaline phosphatase activities of 24.39 and 21.70 $\mu\text{g pNP mL}^{-1} \text{ h}^{-1}$, respectively. Mineralized phytate (6.75 mg L^{-1}), and produced indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) at concentrations of 27.28 and 34.95 $\mu\text{g mL}^{-1}$, respectively. However, its effectiveness as a bioinput for plants when applied to soil/substrate will depend on the nutrient level in the soil, mainly the availability of compatible phosphorus substrates, and the inoculation dose. Therefore, further studies should evaluate its performance under different edaphoclimatic conditions and management strategies to maximize its agronomic efficiency.

Declarations

Ethics, Consent to Participate, and Consent to Publish declarations:

Not applicable.

Clinical trial number

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflicts of interest.

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Author Contribution

Conceptualization: GAS, JS, MDC, NFB and RST; Methodology: GAS, PMSG, HPG, JS, MDC, NFB and RST; Data collection: GAS, PMSG, HPG, FCS and AJB; Data analyses: GAS and RST; Writing original draft: GAS; Review and Editing: All authors. All authors agreed to the final version of this manuscript.

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Data Availability

The data from this study are available upon request to the corresponding author.

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Figures

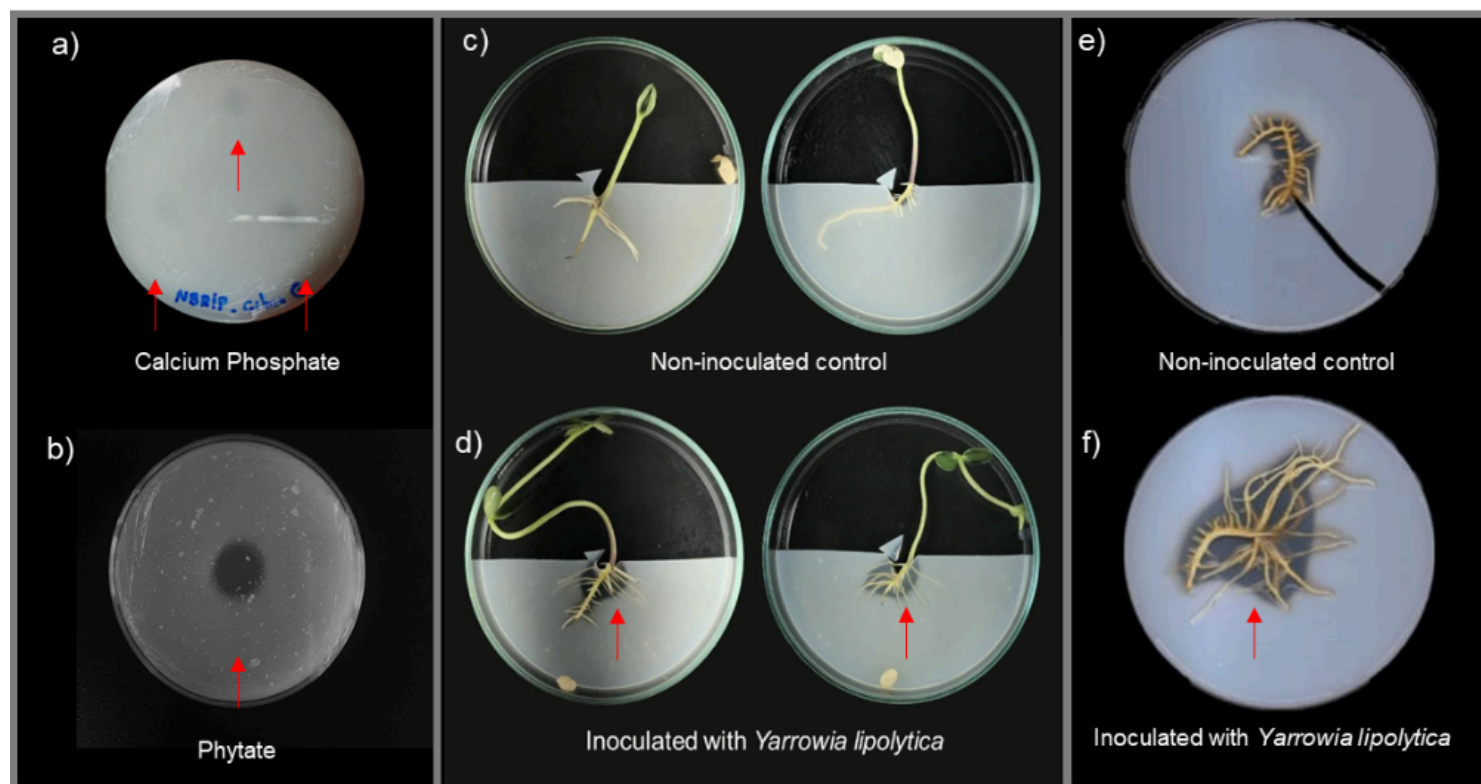


Figure 1

Qualitative assays showing calcium phosphate solubilization by yeast inoculation, evidenced by a slight halo around the inoculation drop (a), and phytate mineralization, evidenced by halo formation in the drop assay (b) and in the rhizosphere of soybean MiniMax plants inoculated with yeast (d and f) compared with non-inoculated plants (c and e). The red arrows indicate halos.

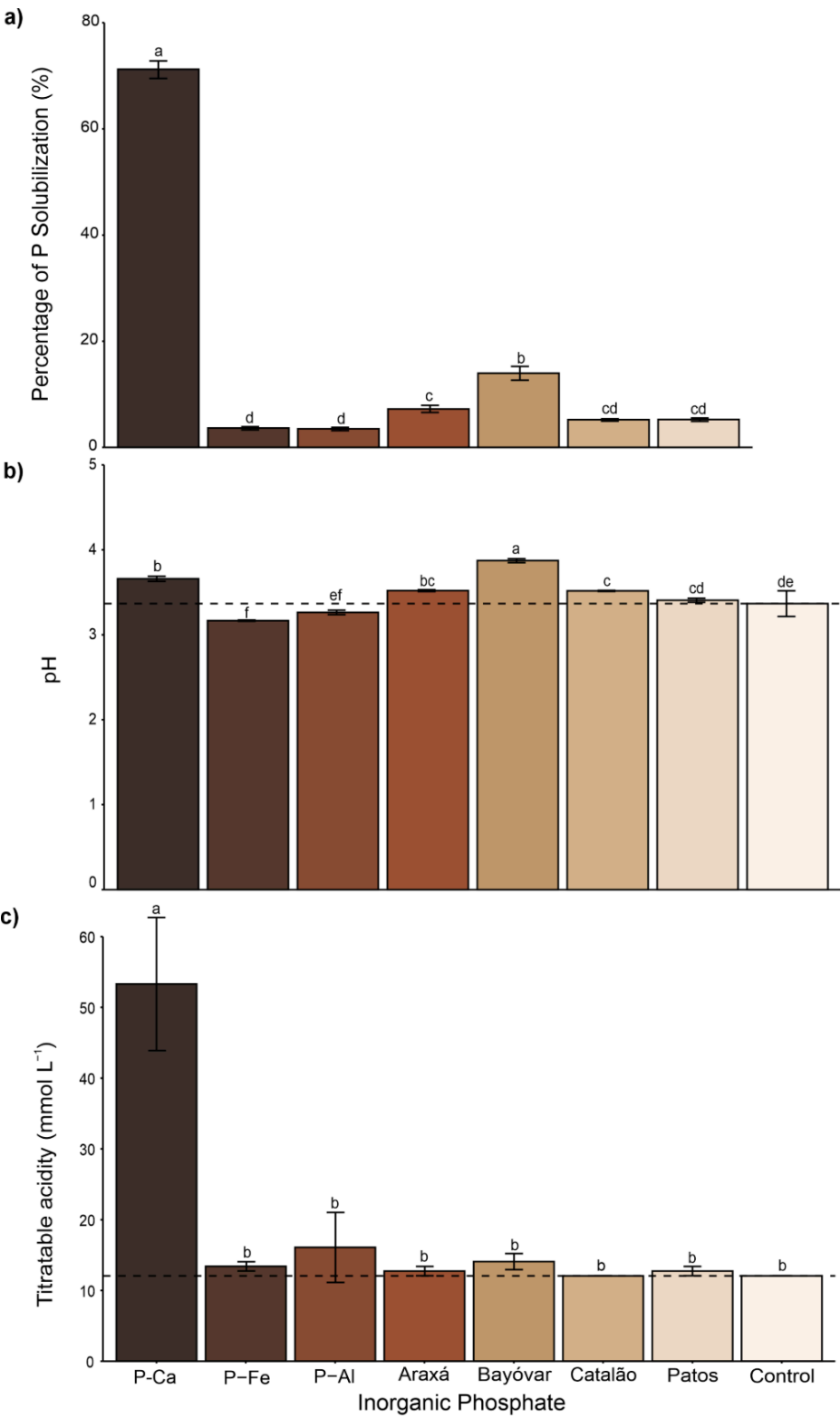


Figure 2

Quantitative test of phosphate solubilization from inorganic phosphates (calcium, iron, and aluminum phosphates) and natural sources (Araxá, Bayóvar, Catalão, and Patos RPs). Phosphate solubilization (%) (a), supernatant pH (b), and titratable acidity (mmol L^{-1}) (c). Dashed lines in (b) and (c) indicate the control treatment. Different letters denote significant differences (Tukey's test, $p < 0.05$) and the lines above the bars represent 95% confidence intervals ($n = 3$).

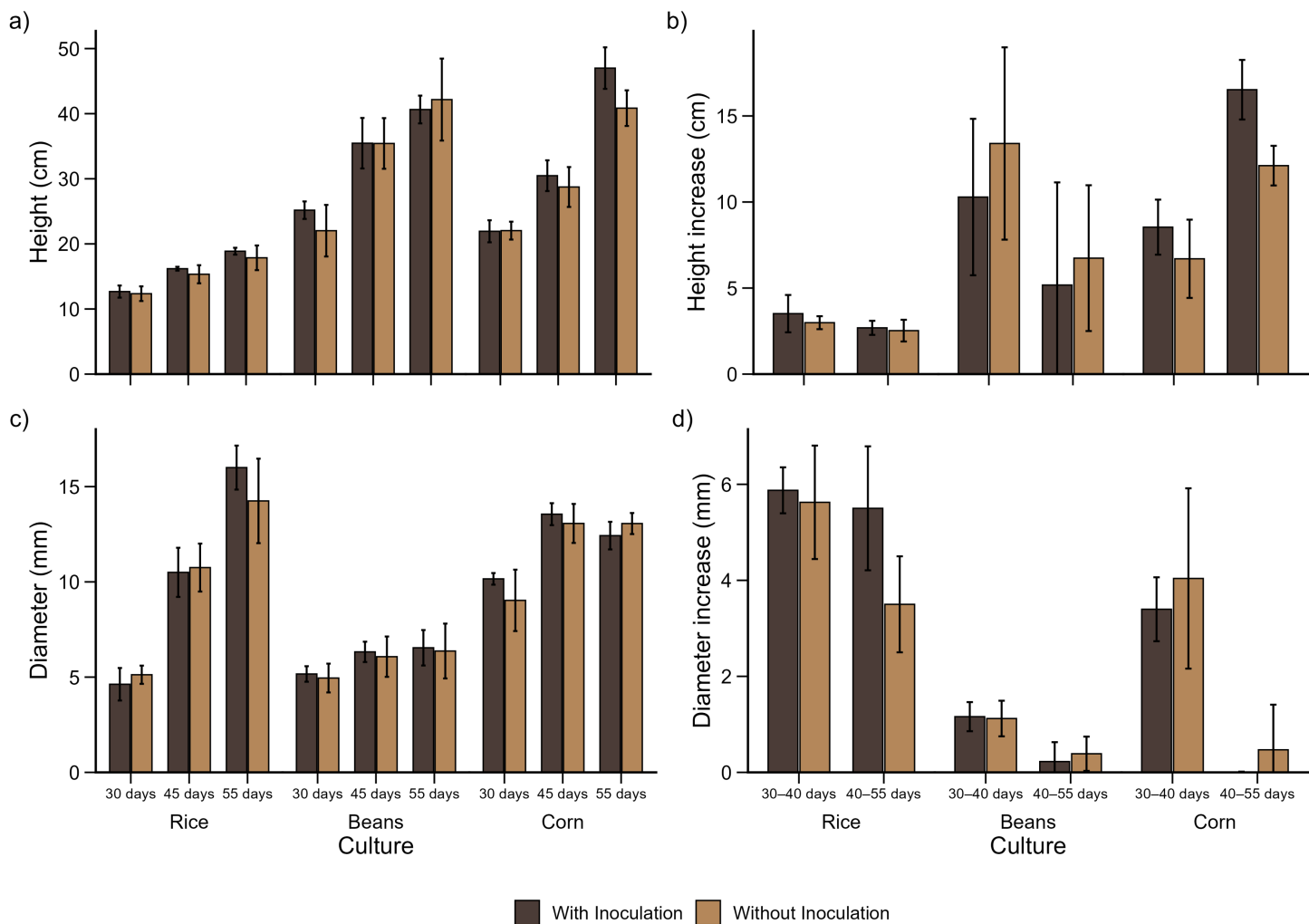


Figure 3

Plant height at 30, 40, and 55 days (a); height increment between 30–40 and 40–55 days (b); stem diameter at 30, 40, and 55 days (c); and stem diameter increment between 30–40 and 40–55 days (d) of rice, bean, and maize plants, under inoculated and non-inoculated conditions. The lines above the bars represent 95% confidence intervals ($n = 4$). Inoculation at the applied dose did not significantly affect any of the evaluated variables in rice, common bean, or maize ($p > 0.05$).

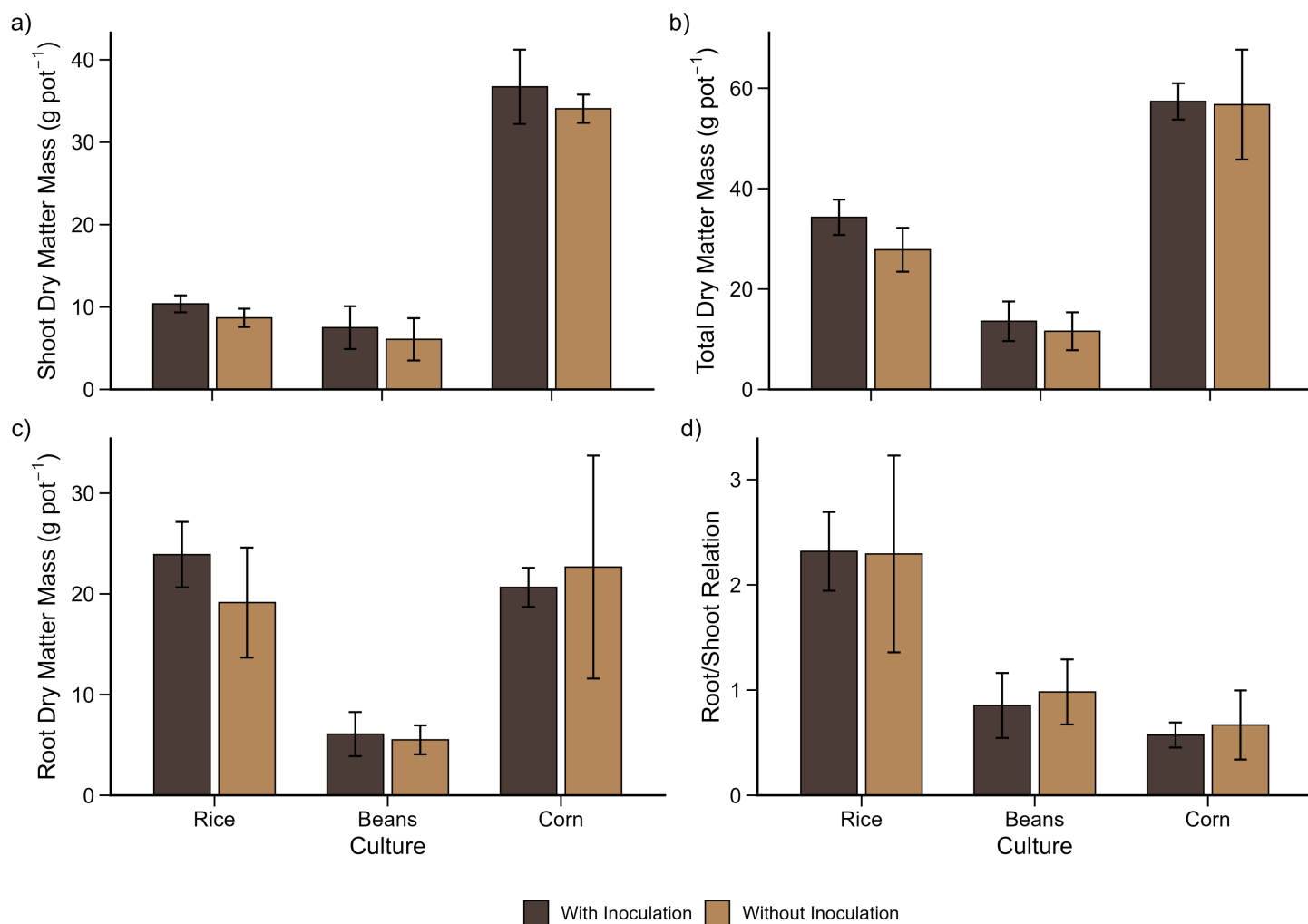


Figure 4

Shoot dry matter mass (a), root dry matter mass (b), total dry matter mass (c), and root/shoot ratio (d) of rice, common bean, and maize plants, under inoculated and non-inoculated conditions. The lines above the bars represent 95% confidence intervals (n = 4). Inoculation at the applied dose did not significantly affect any of the evaluated variables in rice, common bean, or maize ($p > 0.05$).

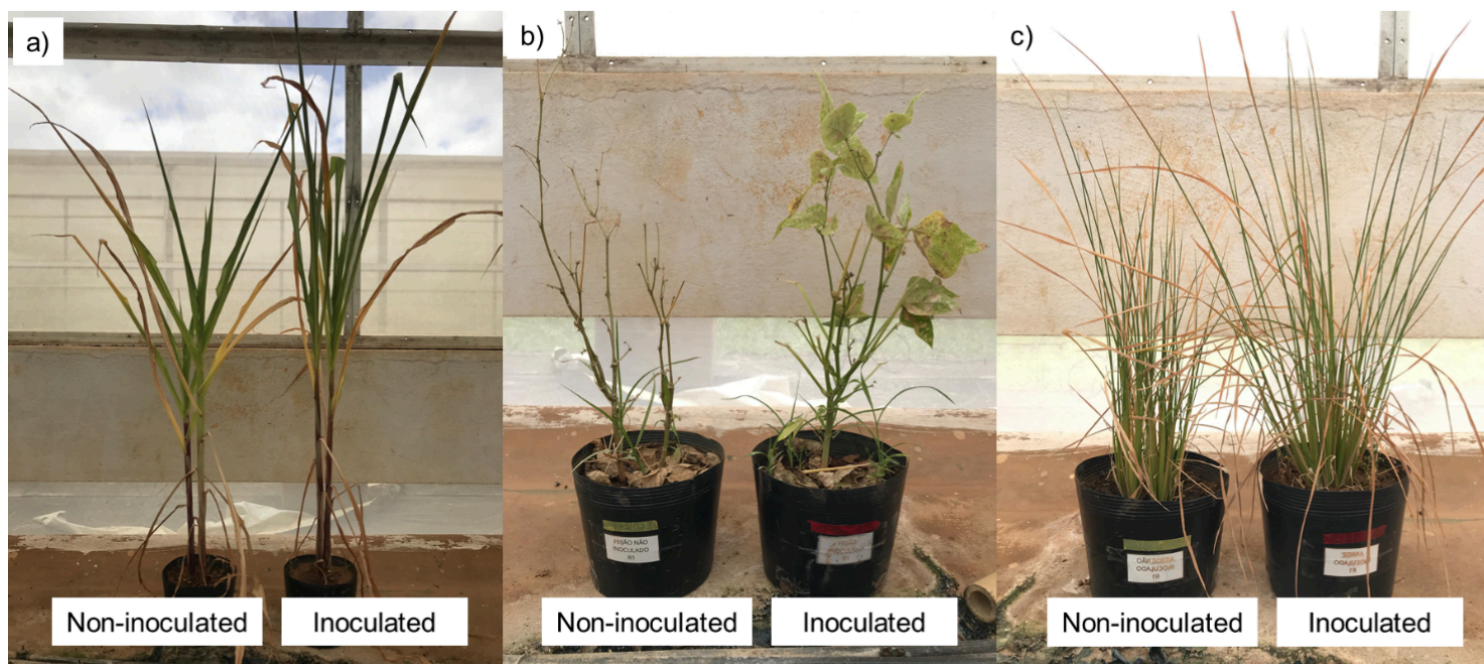


Figure 5

Representative appearance of (a) corn, (b) common bean, and (c) rice plants at 60 days after sowing, grown under greenhouse conditions without inoculation (left) and inoculated with *Yarrowia lipolytica* (right).

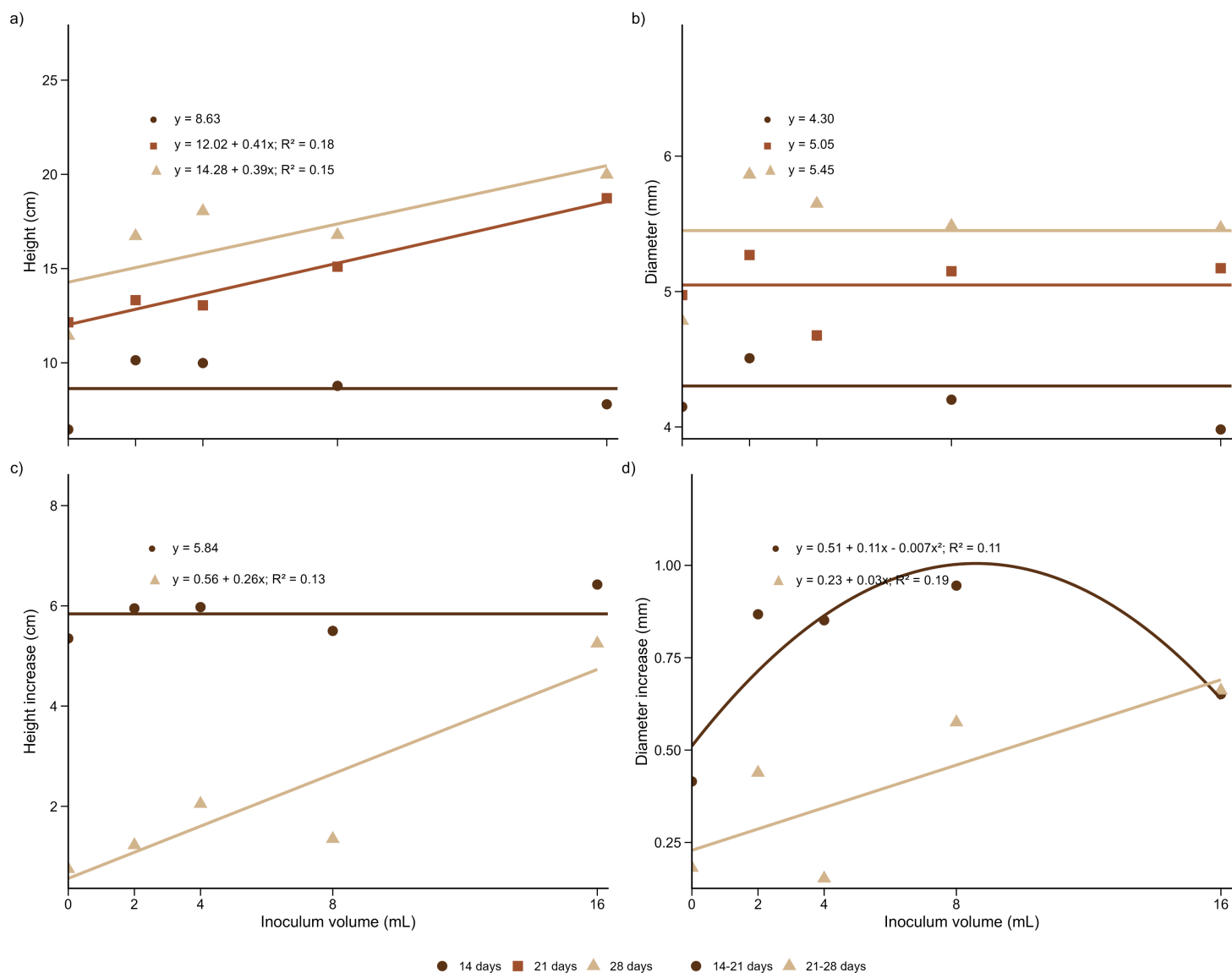


Figure 6

Plant height at 14, 21, and 28 days (a); height increment between 14–21 and 21–28 days (b); stem diameter at 14, 21, and 28 days (c); and stem diameter increment between 14–21 and 21–28 days (d) of MicroTom tomato plants inoculated with 0, 2, 4, 8, and 16 mL of microbial suspension. Symbols represent treatment means (n = 8), and lines represent the fitted regression models. Regression equations are shown only for variables with significant responses to inoculum volume ($p < 0.05$).

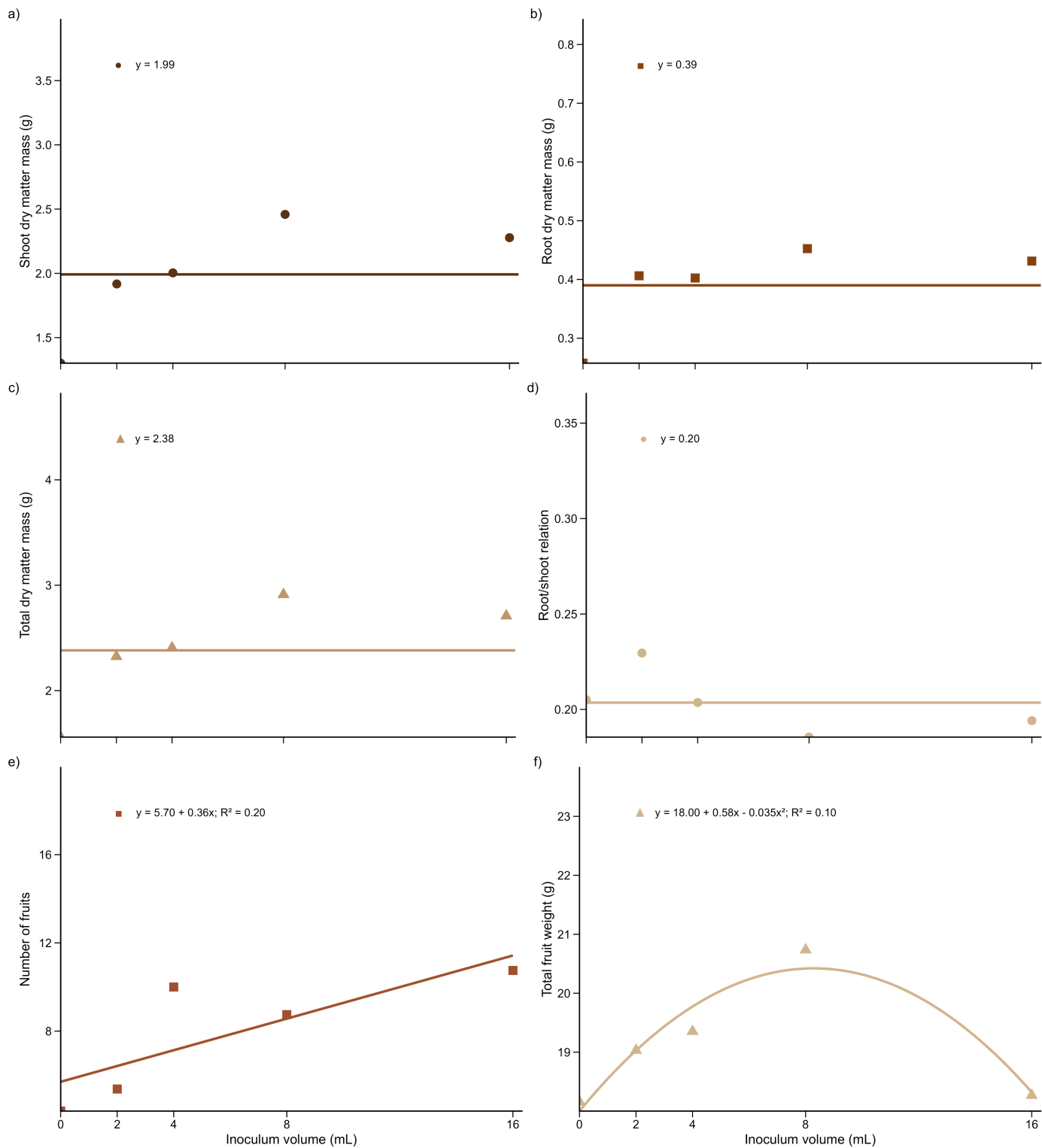


Figure 7

Shoot dry matter mass (a), root dry matter mass (b), total dry matter mass (c), and root/shoot ratio (d), and number of fruits (e), and total fruit weight (f) of *MicroTom* tomato plants inoculated with 0, 2, 4, 8, and 16 mL of microbial suspension. Symbols represent treatment means ($n = 8$), and lines represent the fitted regression models. Regression equations are presented only for significant models ($p < 0.05$).

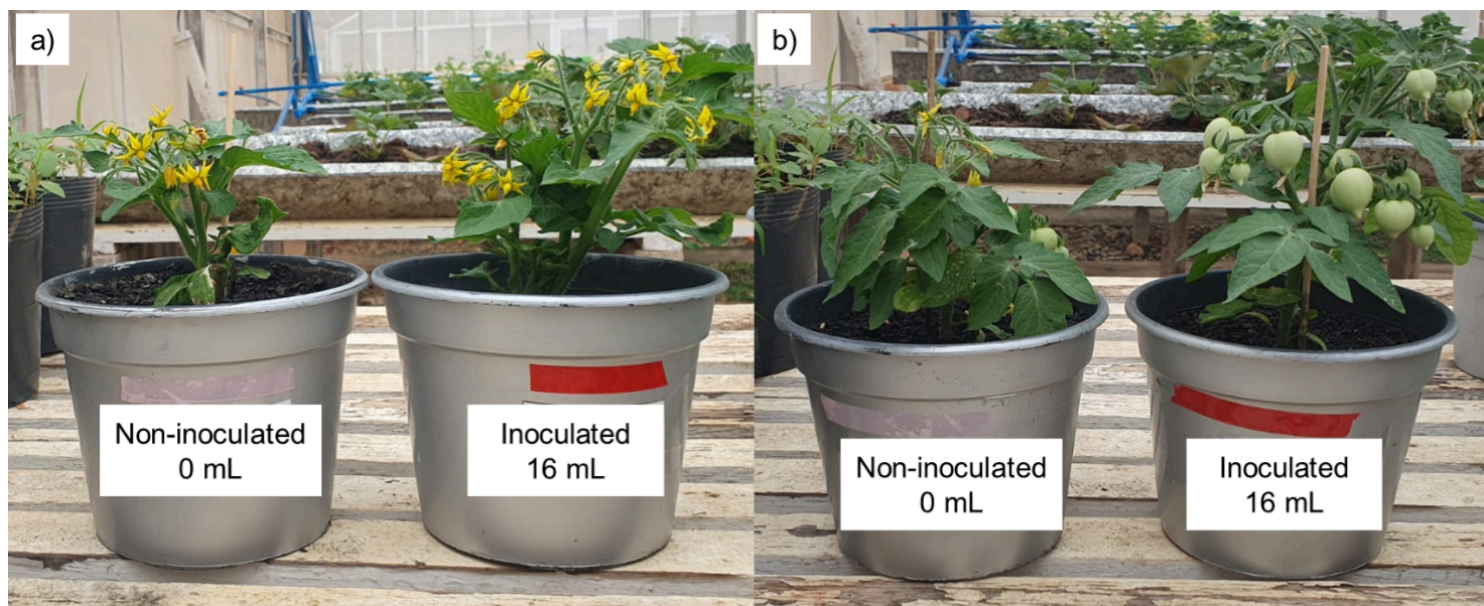


Figure 8

Representative appearance of Micro-Tom tomato plants uninoculated control (0 mL) and inoculated with 16 mL of *Yarrowia lipolytica* suspension under greenhouse conditions, showing (a) the flowering stage and (b) the fruiting stage. Images were taken at the corresponding phenological stages during the experiment.

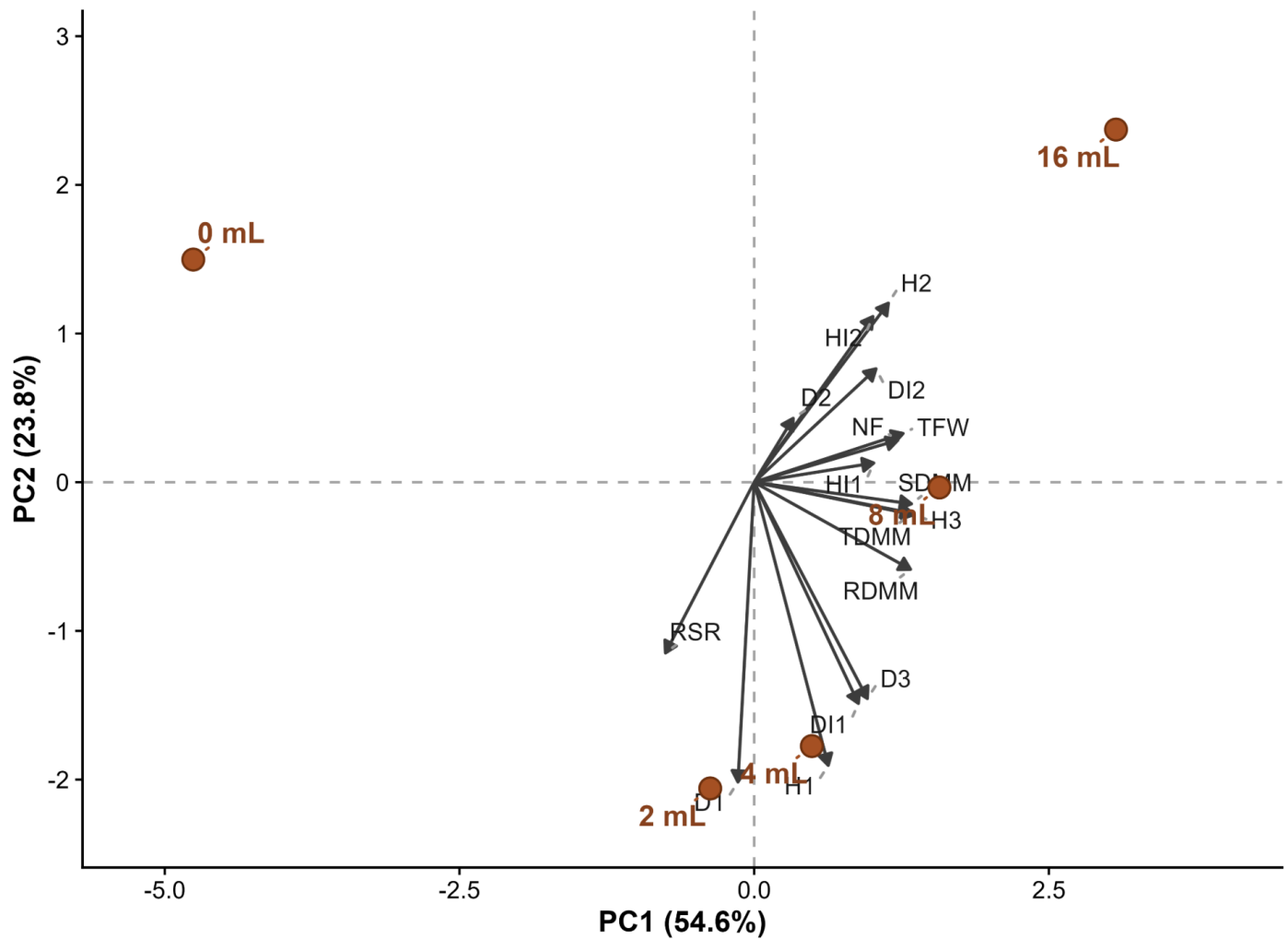


Figure 9

Principal component analysis (PCA) of *in vivo* data from *MicroTom* tomato plants inoculated with 0, 2, 4, 8, and 16 mL of microbial suspension. The analysis summarizes the variation in plant growth traits. Each point represents an individual replicate (n = 8), and vectors indicate the contribution of each trait to the principal components. H: height; D: diameter; HI: height increase; DI: diameter increase; SDMM: shoot dry matter mass; RDMM: root dry matter mass; TDMM: total dry matter mass; RSR: root/shoot relation; NF: number of fruits; TFW: total fruit weight.

Supplementary Files

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